Exhibit B

EXHIBIT B

PATENT
Attorney Docket No. 7681.0010-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Campbell et al.

Serial No.: 09/650,194

Filed: August 29, 2000

For: UNACTIVATED OOCYTES AS CYTOPLAST RECIPIENTS FOR NUCLEAR TRANSFER

Group Art Unit: 1632

Examiner: Unassigned

SUPPORT FOR APPLICANTS' CLAIMS IN THEIR APPLICATION SERIAL NO. 09/650,194

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
19. A method of cloning	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25). Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
a cow	It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. (Page 5, lines 20-24). 8. A method as claimed in any one of claims 1 to 7, wherein the animal is a cow or bull . . . . (Page 29, lines 32-33). According to a fourth aspect of the invention, there is provided an animal prepared as described above. (Page 20, lines 22-23).
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).
(i) inserting a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
of a cultured	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).
diploid bovine fibroblast	Donors which are diploid at the time of transfer are necessary in order to maintain the correct ploidy of the reconstituted embryo. (Page 7, lines 18-20). Table 2 shows pronuclear formation in enucleated oocytes fused to primary bovine fibroblasts (24 hpm) and subsequently activated (42 hpm). (Page 24, lines 2-4).
in the G1 phase of the cell cycle	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24).
into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 6).
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31).
bovine oocyte	Example 1: "MAGIC" Procedure using Bovine Oocytes (Page 21, line 4).
to reconstruct an embryo;	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo . . . . (Page 5, lines 1-3). Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).

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(ii) maintaining the reconstructed embryo without activation	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e., diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.
for a sufficient time to allow the reconstructed embryo to become capable of developing to term;	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12, lines 21-28).
(iii) activating the resultant reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).

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(iv) culturing said activated, reconstructed embryo to blastocyst; and	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
(v) transferring	After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9). The process can be regarded as involving five steps: * * * (5) transfer if necessary to final recipient. (Page 19, lines 14-28).
said cultured, reconstructed embryo	These results show that: * * * iii) reconstructed embryos or enucleated pulsed oocytes can be cultured in maturation medium and do not undergo spontaneous activation; (Page 24, line 32- page 25, line 12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
to a host cow	It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. (Page 5, lines 20-24). 8. A method as claimed in any one of claims 1 to 7, wherein the animal is a cow or bull . . . . (Page 29, lines 32-33).
such that the reconstructed embryo develops to term.	According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 15, lines 19-27). The second step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. (Page 15, lines 31-33). According to a fourth aspect of the invention, there is provided an animal prepared as described above. (Page 20, lines 22-23).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
20. The method of claim 19, wherein said step of activating the resultant reconstructed embryo comprises activating the embryo with a DC pulse.	Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5 µg/ml) in M2 medium for 1 hour prior to activation and 5 hours post activation. Couplets were activated with a single DC pulse of 1.25 Kv/cm for 80µsec. (Page 26, lines 4-12).
21. The method of claim 19 or 20, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vitro*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). If *in vitro* conditions are used, those routinely employed in the art are quite acceptable. (Page 17, lines 26-27).

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22. The method of claim 19 or 20, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vivo*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). Experience suggests that embryos derived by nuclear transfer are different from normal embryos and sometimes benefit from or even require culture conditions *in vivo* other than those in which embryos are usually cultured (at least *in vivo*). The reason for this is not known. (Page 17, lines 5-9).
23. A method of cloning	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12).

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	13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25). Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
a bovine fetus	It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. (Page 5, lines 20-24). According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 15, lines 19-27). The second step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. (Page 15, lines 31-33). After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9).
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).

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(i) inserting a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).
of a cultured	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).
diploid bovine fibroblast	Donors which are diploid at the time of transfer are necessary in order to maintain the correct ploidy of the reconstituted embryo. (Page 7, lines 18-20). Table 2 shows pronuclear formation in enucleated oocytes fused to primary bovine fibroblasts (24 hpm) and subsequently activated (42 hpm). (Page 24, lines 2-4).
in the G1 phase of the cell cycle	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24).

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into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21).
	In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 6).
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31).
bovine oocyte	Example 1: "MAGIC" Procedure using Bovine Oocytes (Page 21, line 4).

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to reconstruct an embryo;	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo . . . . (Page 5, lines 1-3). Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).
(ii) maintaining the reconstructed embryo without activation	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e., diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.
for a sufficient time to allow the reconstructed embryo to become capable of developing to term;	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12, lines 21-28).

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(iii) activating the resultant reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).
(iv) culturing said activated, reconstructed embryo to blastocyst; and	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
(v) transferring	After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9). The process can be regarded as involving five steps: * * * (5) transfer if necessary to final recipient. (Page 19, lines 14-28).

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said cultured, reconstructed embryo	These results show that: * * * iii) reconstructed embryos or enucleated pulsed oocytes can be cultured in maturation medium and do not undergo spontaneous activation; (Page 24, line 32- page 25, line 12).
to a host cow	It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. (Page 5, lines 20-24). 8. A method as claimed in any one of claims 1 to 7, wherein the animal is a cow or bull . . . . (Page 29, lines 32-33).

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such that the reconstructed embryo develops into a fetus.	According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 15, lines 19-27). The second step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. (Page 15, lines 31-33). After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9).
24. The method of claim 23, wherein said step of activating the resultant reconstructed embryo comprises activating the embryo with a DC pulse.	Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5 µg/ml) in M2 medium for 1 hour prior to activation and 5 hours post activation. Couplets were activated with a single DC pulse of 1.25 Kv/cm for 80µsec. (Page 26, lines 4-12).

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25. The method of claim 23 or 24, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vitro*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). If *in vitro* conditions are used, those routinely employed in the art are quite acceptable. (Page 17, lines 26-27).
26. The method of claim 23 or 24, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vivo*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). Experience suggests that embryos derived by nuclear transfer are different from normal embryos and sometimes benefit from or even require culture conditions *in vivo* other than those in which embryos are usually cultured (at least *in vivo*). The reason for this is not known. (Page 17, lines 5-9).
27. A method of cloning	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12).

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	13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25). Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1).

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a sheep	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).
(i) inserting a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
of a cultured	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).
diploid ovine fibroblast	Table 2 shows pronuclear formation in enucleated oocytes fused to primary bovine fibroblasts (24 hpm) and subsequently activated (42 hpm). (Page 24, lines 2-4). Similar observations to those in Example 1 have also been made in ovine oocytes which have been matured *in vivo*. (Page 26, lines 20-21).
in the G1 phase of the cell cycle	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24).
into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21).

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	In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 6).
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31).
ovine oocyte	Similar observations to those in Example 1 have also been made in ovine oocytes which have been matured *in vivo*. (Page 26, lines 20-21).
to reconstruct an embryo;	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo . . . . (Page 5, lines 1-3). Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).

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(ii) maintaining the reconstructed embryo without activation	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e., diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.
for a sufficient time to allow the reconstructed embryo to become capable of developing to term;	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12, lines 21-28).
(iii) activating the resultant reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).

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(iv) culturing said activated, reconstructed embryo to blastocyst; and	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
(v) transferring	After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9). The process can be regarded as involving five steps: * * * (5) transfer if necessary to final recipient. (Page 19, lines 14-28).
said cultured, reconstructed embryo	These results show that: * * * iii) reconstructed embryos or enucleated pulsed oocytes can be cultured in maturation medium and do not undergo spontaneous activation; (Page 24, line 32- page 25, line 12).

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to a host sheep	Reconstructed embryos were cultured in the ligated oviduct of a temporary recipient ewe for 6 days, recovered and assessed for development. (Page 27, lines 12-14).
such that the reconstructed embryo develops to term.	In preliminary experiments in sheep, a single pregnancy has resulted in the birth of single live lamb. The results are summarised in tables 4 and 5. (Page 27, line. 2-4).
28. The method of claim 27, wherein said step of activating the resultant reconstructed embryo comprises activating the embryo with a DC pulse.	Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5 µg/ml) in M2 medium for 1 hour prior to activation and 5 hours post activation. Couplets were activated with a single DC pulse of 1.25 Kv/cm for 80µsec. (Page 26, lines 4-12).
29. The method of claim 27 or 28, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vitro*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). If *in vitro* conditions are used, those routinely employed in the art are quite acceptable. (Page 17, lines 26-27).

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30. The method of claim 27 or 28, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vivo*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). Experience suggests that embryos derived by nuclear transfer are different from normal embryos and sometimes benefit from or even require culture conditions *in vivo* other than those in which embryos are usually cultured (at least *in vivo*). The reason for this is not known. (Page 17, lines 5-9).
31. A method of cloning	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25). Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
a ovine fetus	Similar observations to those in Example 1 have also been made in ovine oocytes which have been matured *in vivo*. (Page 26, lines 20-21). According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).
(i) inserting a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
of a cultured	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).
diploid ovine fibroblast	Table 2 shows pronuclear formation in enucleated oocytes fused to primary bovine fibroblasts (24 hpm) and subsequently activated (42 hpm). (Page 24, lines 2-4). Similar observations to those in Example 1 have also been made in ovine oocytes which have been matured *in vivo*. (Page 26, lines 20-21).
in the G1 phase of the cell cycle	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24).
into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 6).
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31).
ovine oocyte	Table 2 shows pronuclear formation in enucleated oocytes fused to primary bovine fibroblasts (24 hpm) and subsequently activated (42 hpm). (Page 24, lines 2-4). Similar observations to those in Example 1 have also been made in ovine oocytes which have been matured *in vivo*. (Page 26, lines 20-21).
to reconstruct an embryo;	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo . . . . (Page 5, lines 1-3). Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
(ii) maintaining the reconstructed embryo without activation	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e., diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.
for a sufficient time to allow the reconstructed embryo to become capable of developing to term;	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12, lines 21-28).
(iii) activating the resultant reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
(iv) culturing said activated, reconstructed embryo to blastocyst; and	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
(v) transferring	After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9). The process can be regarded as involving five steps: * * * (5) transfer if necessary to final recipient. (Page 19, lines 14-28).
said cultured, reconstructed embryo	These results show that: * * * iii) reconstructed embryos or enucleated pulsed oocytes can be cultured in maturation medium and do not undergo spontaneous activation; (Page 24, line 32- page 25, line 12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
to a host sheep	39. Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).
such that the reconstructed embryo develops into a fetus	40. The table shows the total number of embryos from each group transferred the frequency of pregnancy in terms of ewes and embryos, in the majority of cases 2 embryos were transferred to each ewe. (Page 28, lines 4-7). Table 5 * * * Total Number Ewes 6 Pregnant Ewes % 1 (16.7) Foetuses/Total Transferred (%) 2/10 (20.0)
32. The method of claim 31, wherein said step of activating the resultant reconstructed embryo comprises activating the embryo with a DC pulse.	Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5 µg/ml) in M2 medium for 1 hour prior to activation and 5 hours post activation. Couplets were activated with a single DC pulse of 1.25 Kv/cm for 80µsec. (Page 26, lines 4-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
33. The method of claim 31 or 32, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vitro*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). If *in vitro* conditions are used, those routinely employed in the art are quite acceptable. (Page 17, lines 26-27).
34. The method of claim 31 or 32, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vivo*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). Experience suggests that embryos derived by nuclear transfer are different from normal embryos and sometimes benefit from or even require culture conditions *in vivo* other than those in which embryos are usually cultured (at least *in vivo*). The reason for this is not known. (Page 17, lines 5-9).
35. A method of cloning	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25). Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1).
a non-human mammal	Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. (Page 8, lines 13-19).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).
(i) inserting a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).
of a cultured	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
diploid non-human mammalian fibroblast	Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. (Page 8, lines 13-19).
in the G1 phase of the cell cycle	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24).
into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21).
	In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 6).
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
non-human mammalian oocyte	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28). According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).
of the same species	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
to reconstruct an embryo;	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo . . . . (Page 5, lines 1-3). Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).
(ii) maintaining the reconstructed embryo without activation	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e., diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.
for a sufficient time to allow the reconstructed embryo to become capable of developing to term;	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12, lines 21-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
(iii) activating the resultant reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).
(iv) culturing said activated, reconstructed embryo to blastocyst; and	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
(v) transferring	After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9). The process can be regarded as involving five steps: * * * (5) transfer if necessary to final recipient. (Page 19, lines 14-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
said cultured, reconstructed embryo	These results show that: * * * iii) reconstructed embryos or enucleated pulsed oocytes can be cultured in maturation medium and do not undergo spontaneous activation; (Page 24, line 32- page 25, line 12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
to a host non-human mammal	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28). According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).
of the same species	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
such that the reconstructed embryo develops to term.	According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 15, lines 19-27). The second step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. (Page 15, lines 31-33). According to a fourth aspect of the invention, there is provided an animal prepared as described above. (Page 20, lines 22-23).
36. The method of claim 35, wherein said step of activating the resultant reconstructed embryo comprises activating the embryo with a DC pulse.	Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5 µg/ml) in M2 medium for 1 hour prior to activation and 5 hours post activation. Couplets were activated with a single DC pulse of 1.25 Kv/cm for 80µsec. (Page 26, lines 4-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
37. The method of claim 35 or 36, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vitro*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). If *in vitro* conditions are used, those routinely employed in the art are quite acceptable. (Page 17, lines 26-27).
38. The method of claim 35 or 36, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vivo*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). Experience suggests that embryos derived by nuclear transfer are different from normal embryos and sometimes benefit from or even require culture conditions *in vivo* other than those in which embryos are usually cultured (at least *in vivo*). The reason for this is not known. (Page 17, lines 5-9).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
39. A method of cloning	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12). 13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1).
a non-human mammaliam	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
fetus	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).
(i) inserting a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
Of a cultured	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).
diploid non-human mammalian fibroblast	Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. (Page 8, lines 13-19).
in the G1 phase of the cell cycle	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24).
into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 6).
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
non-human mammalian oocyte	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28). According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).
of the same species	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
to reconstruct an embryo;	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo . . . . (Page 5, lines 1-3). Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).
(ii) maintaining the reconstructed embryo without activation	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e., diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.
for a sufficient time to allow the reconstructed embryo to become capable of developing to term;	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12, lines 21-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
(iii) activating the resultant reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).
(iv) culturing said activated, reconstructed embryo to blastocyst; and	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
(v) transferring	After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9). The process can be regarded as involving five steps: * * * (5) transfer if necessary to final recipient. (Page 19, lines 14-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
said cultured, reconstructed embryo	These results show that: * * * iii) reconstructed embryos or enucleated pulsed oocytes can be cultured in maturation medium and do not undergo spontaneous activation; (Page 24, line 32- page 25, line 12).
to a host non-human mammal	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).
of the same species	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
such that the reconstructed embryo develops into a fetus.	According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 15, lines 19-27). The second step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. (Page 15, lines 31-33). After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9).
40. The method of claim 39, wherein said step of activating the resultant reconstructed embryo comprises activating the embryo with a DC pulse.	Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5 µg/ml) in M2 medium for 1 hour prior to activation and 5 hours post activation. Couplets were activated with a single DC pulse of 1.25 Kv/cm for 80µsec. (Page 26, lines 4-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
41. The method of claim 39 or 40, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vitro*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). If *in vitro* conditions are used, those routinely employed in the art are quite acceptable. (Page 17, lines 26-27).
42. The method of claim 39 or 40, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vivo*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). Experience suggests that embryos derived by nuclear transfer are different from normal embryos and sometimes benefit from or even require culture conditions *in vivo* other than those in which embryos are usually cultured (at least *in vivo*). The reason for this is not known. (Page 17, lines 5-9).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
43. A method of cloning	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12). 13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1).
a non-human mammal	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).
(i) inserting a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).
of a cultured	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
diploid non-human mammalian differentiated cell	Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. (Page 8, lines 13-19).
in the G1 phase of the cell cycle	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24).
into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21).
	In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 6).
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
non-human mammalian oocyte	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28). According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).
of the same species	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
to reconstruct an embryo;	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo . . . . (Page 5, lines 1-3). Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).
(ii) maintaining the reconstructed embryo without activation	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e., diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.
for a sufficient time to allow the reconstructed embryo to become capable of developing to term;	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12, lines 21-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
(iii) activating the resultant reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).
(iv) culturing said activated, reconstructed embryo to blastocyst; and	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
(v) transferring	After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9). The process can be regarded as involving five steps: * * * (5) transfer if necessary to final recipient. (Page 19, lines 14-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
said cultured, reconstructed embryo	These results show that: * * * iii) reconstructed embryos or enucleated pulsed oocytes can be cultured in maturation medium and do not undergo spontaneous activation; (Page 24, line 32- page 25, line 12).
to a host non-human mammal	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).
of the same species	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
such that the reconstructed embryo develops to term.	According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 15, lines 19-27). The second step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. (Page 15, lines 31-33). According to a fourth aspect of the invention, there is provided an animal prepared as described above. (Page 20, lines 22-23).
44. The method of claim 43, wherein said step of activating the resultant reconstructed embryo comprises activating the embryo with a DC pulse.	Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5 µg/ml) in M2 medium for 1 hour prior to activation and 5 hours post activation. Couplets were activated with a single DC pulse of 1.25 Kv/cm for 80µsec. (Page 26, lines 4-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
45. The method of claim 43 or 44, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vitro*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). If *in vitro* conditions are used, those routinely employed in the art are quite acceptable. (Page 17, lines 26-27).
46. The method of claim 43 or 44, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vivo*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). Experience suggests that embryos derived by nuclear transfer are different from normal embryos and sometimes benefit from or even require culture conditions *in vivo* other than those in which embryos are usually cultured (at least *in vivo*). The reason for this is not known. (Page 17, lines 5-9).
47. A method of cloning	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
	13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25). Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
a non-human mammalian	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).
fetus	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).
(i) inserting a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).
of a cultured	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
diploid non-human mammalian differentiated cell	Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. (Page 8, lines 13-19).
in the G1 phase of the cell cycle	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24).
into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21).
	In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 6).
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
non-human mammalian oocyte	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28). According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).
of the same species	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
to reconstruct an embryo;	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo . . . . (Page 5, lines 1-3). Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn of synchronised final recipient blackface ewes. (Page 28, lines 1-4).
(ii) maintaining the reconstructed embryo without activation	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). It has now been found that nuclear transfer into an oocyte arrested in metaphase II can give rise to a viable embryo if normal ploidy (i.e., diploidy) is maintained and if the embryo is not activated at the time of nuclear transfer. The delay in activation allows the nucleus to remain exposed to the recipient cytoplasm.
for a sufficient time to allow the reconstructed embryo to become capable of developing to term;	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12, lines 21-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
(iii) activating the resultant reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).
(iv) culturing said activated, reconstructed embryo to blastocyst; and	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
(v) transferring	After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9). The process can be regarded as involving five steps: * * * (5) transfer if necessary to final recipient. (Page 19, lines 14-28).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
said cultured, reconstructed embryo	These results show that: * * * iii) reconstructed embryos or enucleated pulsed oocytes can be cultured in maturation medium and do not undergo spontaneous activation; (Page 24, line 32- page 25, line 12).
to a host non-human mammal	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).
of the same species	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31.)

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
such that the reconstructed embryo develops into a fetus	According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 15, lines 19-27). The second step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. (Page 15, lines 31-33). After screening, if screening, has taken place, the blastocyst embryo is allowed to develop to term. This will generally be *in vivo*. If development up to blastocyst has taken place *in vitro*, then transfer into the final recipient animal takes place at this stage. (Page 18, lines 5-9).
48. The method of claim 47, wherein said step of activating the resultant reconstructed embryo comprises activating the embryo with a DC pulse.	Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5 µg/ml) in M2 medium for 1 hour prior to activation and 5 hours post activation. Couplets were activated with a single DC pulse of 1.25 Kv/cm for 80µsec. (Page 26, lines 4-12).

Limitations in Campbell et al.'s claims	Support in Application S.N. 09/650,194
49. The method of claim 47 or 48, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vitro*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). If *in vitro* conditions are used, those routinely employed in the art are quite acceptable. (Page 17, lines 26-27).
50. The method of claim 47 or 48, wherein said step of culturing said activated, reconstructed embryo to blastocyst is performed *in vivo*.	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). Experience suggests that embryos derived by nuclear transfer are different from normal embryos and sometimes benefit from or even require culture conditions *in vivo* other than those in which embryos are usually cultured (at least *in vivo*). The reason for this is not known. (Page 17, lines 5-9).